

Accepted Manuscript

Hepatitis delta-associated mortality in HIV/HBV-coinfected patients

Charles Béguelin, Darius Moradpour, Roland Sahli, Franziska Suter-Riniker, Alexander Lüthi, Matthias Cavassini, Huldrych F. Günthard, Manuel Battegay, Enos Bernasconi, Patrick Schmid, Alexandra Calmy, Dominique Braun, Hansjakob Furrer, Andri Rauch, Gilles Wandeler, the Swiss HIV Cohort Study,

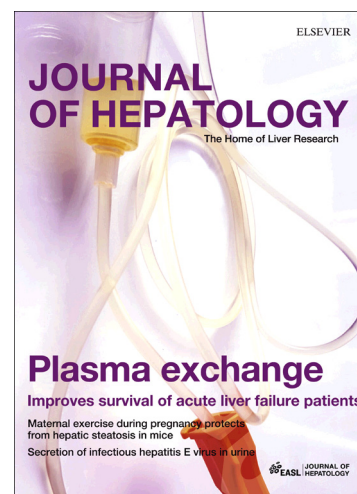
PII: S0168-8278(16)30569-4
DOI: <http://dx.doi.org/10.1016/j.jhep.2016.10.007>
Reference: JHEPAT 6285

To appear in: *Journal of Hepatology*

Received Date: 10 May 2016
Revised Date: 28 September 2016
Accepted Date: 3 October 2016

Please cite this article as: Béguelin, C., Moradpour, D., Sahli, R., Suter-Riniker, F., Lüthi, A., Cavassini, M., Günthard, H.F., Battegay, M., Bernasconi, E., Schmid, P., Calmy, A., Braun, D., Furrer, H., Rauch, A., Wandeler, G., the Swiss HIV Cohort Study, Hepatitis delta-associated mortality in HIV/HBV-coinfected patients, *Journal of Hepatology* (2016), doi: <http://dx.doi.org/10.1016/j.jhep.2016.10.007>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



Hepatitis delta-associated mortality in HIV/HBV-coinfected patients

Charles Béguelin¹, Darius Moradpour², Roland Sahli³, Franziska Suter-Riniker⁴, Alexander Lüthi⁴, Matthias Cavassini⁵, Huldrych F. Günthard^{6,7}, Manuel Battegay⁸, Enos Bernasconi⁹, Patrick Schmid¹⁰, Alexandra Calmy¹¹, Dominique Braun^{6,7}, Hansjakob Furrer¹, Andri Rauch^{1*}, Gilles Wandeler^{1,12*} and the Swiss HIV Cohort Study

*contributed equally

¹Department of Infectious Diseases, Bern University Hospital, University of Bern, Switzerland, ²Division of Gastroenterology and Hepatology, Centre Hospitalier Universitaire Vaudois, University of Lausanne, ³Institute of Microbiology, Centre Hospitalier Universitaire Vaudois, University of Lausanne, ⁴Institute for Infectious Diseases, Faculty of Medicine, University of Bern, Switzerland, ⁵Division of Infectious Diseases, University Hospital Lausanne, University of Lausanne, ⁶Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, ⁷Institute of Medical Virology, University of Zurich, ⁸Department of Infectious Diseases & Hospital Hygiene, University Hospital Basel, ⁹Division of Infectious Diseases, Regional Hospital Lugano, ¹⁰Division of Infectious Diseases and Hospital Epidemiology, Cantonal Hospital St.Gallen, ¹¹Division of Infectious Diseases, University Hospital Geneva, University of Geneva, ¹²Institute of Social and Preventive Medicine, University of Bern

Corresponding Author:

Dr. med. Gilles Wandeler

Department of Infectious Diseases, Inselspital, Bern University Hospital, University of Bern
Poliklinik Trakt 2B
3010 Bern
Switzerland

E-Mail: gilles.wandeler@ispm.unibe.ch

Tel: +41 31 632 21 11

Word count: 2742

Number of tables: 1

Number of figures: 4

Abbreviations: HDV, hepatitis D virus; HBV, hepatitis B virus; HIV, human immunodeficiency virus; SHCS, Swiss HIV Cohort Study; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; SSA, sub-Saharan Africa; TDF, tenofovir disoproxil fumarate; ART, antiretroviral therapy; OD, optical density; CI, confidence interval; IQR, interquartile range; HCV, hepatitis C virus; PWID, people who inject drugs; ALT, alanine aminotransferase; aHR, adjusted hazard ratio; HET, heterosexual; CDC: Centers for Disease Control; eGFR, estimated glomerular filtration rate; AST, aspartate aminotransferase; Ab: antibody; APRI: AST-to-platelet ratio index

Keywords: hepatitis delta virus, human immunodeficiency virus, coinfection, prevalence, mortality, clinical, outcome

Conflicts of interest: None declared.

Financial support: This study has been financed within the framework of the Swiss HIV Cohort Study, supported by the Swiss National Science Foundation (grant #148522), by SHCS project #769 and by the SHCS research foundation. GW was supported by an Ambizione-PROSPER fellowship from the Swiss National Science Foundation (PZ00P3_154730). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author's contributions: CB, AR and GW conceived and designed the study. CB and GW performed the statistical analyses. CB, AR and GW wrote the first draft of the manuscript. RS performed the molecular HDV analyses. All authors contributed to the acquisition and the interpretation of the data, critically revised the paper and approved its final version.

Abstract (250 words)**Background and aims:**

Hepatitis delta virus (HDV) infection accelerates the progression of hepatitis B virus (HBV)-related liver disease. We assessed the epidemiological characteristics of HDV infection in the nationwide Swiss HIV Cohort Study and evaluated its impact on clinical outcomes.

Methods:

All HIV-infected patients with a positive HBsAg test were considered and tested for anti-HDV antibodies. HDV amplification and sequencing were performed in anti-HDV-positive patients. Demographic and clinical characteristics at initiation of antiretroviral therapy, as well as causes of death were compared between HDV-positive and HDV-negative individuals using descriptive statistics. Kaplan-Meier and multivariable Cox regression analyses were used to evaluate the association between HDV infection and overall mortality, liver-related mortality as well as incidence of hepatocellular carcinoma (HCC).

Results: Of 818 patients with a positive HBsAg, 771 (94%) had a stored serum sample available and were included. The prevalence of HDV infection was 15.4% (119/771, 95% confidence interval (CI): 12.9-18.0) and the proportion of HDV-positive patients with HDV replication 62.9% (73/116). HDV-infected patients were more likely to be persons who inject drugs (PWID) (60.6% vs. 9.1%) and to have a positive HCV serology (73.1% vs. 17.8%) compared to HDV-uninfected ones. HDV infection was strongly associated with overall death (adjusted hazard ratio 2.33, 95% CI 1.41-3.84), liver-related death (7.71, 3.13-18.97) and with the occurrence of HCC (9.30, 3.03-28.61). Results were similar when PWID or HCV-coinfected patients were excluded from the analyses.

Conclusions: The prevalence of HDV in HBsAg-positive patients in the SHCS is high and HDV infection is independently associated with mortality and liver-related events, including HCC.

Introduction:

Worldwide 15-20 million people are infected with hepatitis delta virus (HDV), with the highest prevalence reported in regions endemic for hepatitis B virus (HBV) infection such as Eastern and Mediterranean Europe, sub-Saharan Africa (SSA) and parts of Asia ^{1,2}. It is estimated that 5- 20% of HBV-infected individuals have serological evidence of exposure to HDV ³. However, data on the prevalence of HDV in HIV/HBV-coinfected patients are scarce. Of a selected sample of 422 hepatitis B surface antigen (HBsAg) carriers in the EuroSIDA collaboration, 61 (14%) were coinfecting with HDV ⁴. The prevalence of anti-HDV antibodies seems even higher in HIV/HBV-coinfected patients from SSA, reaching 25% in Guinea-Bissau ⁵. Although the prevalence of HDV infection seems to decrease in some regions, most probably due to HBV preventive measures ⁶, this is not the case in Northern Europe, where most cases are now diagnosed in young migrants from endemic regions ².

HDV accelerates the course of HBV-related liver disease, including the progression to liver cirrhosis, hepatic decompensation and possibly hepatocellular carcinoma (HCC) ^{7,8}. In HIV-infected patients, the presence of HDV-coinfection has been associated with a higher incidence of hepatic flares and decompensation as well as an increased mortality ^{9,10}. However, most studies assessing the impact of HDV on long-term outcomes in HIV/HBV-coinfected individuals were limited by small sample sizes, highly selected study populations, short follow-up periods or by a retrospective design. Although most HIV/HBV/HDV-coinfected patients now experience HBV virological suppression on tenofovir disoproxil fumarate (TDF)-containing antiretroviral therapy (ART), its impact on HDV replication remains controversial ^{11,12}. Thus, its impact on mortality and liver-related complications needs to be evaluated in prospective studies with long-term follow-up.

In this study, we aimed to describe the main epidemiological characteristics of HDV infection and to evaluate its impact on clinical outcomes in the Swiss HIV Cohort Study (SHCS) ¹³. The systematic ascertainment of death causes and liver-related complications in the SHCS allowed us to perform a detailed assessment of liver-related outcomes in a nationwide representative cohort of HIV/HBV-coinfected individuals.

Patients and methods

Swiss HIV Cohort Study (SHCS):

The SHCS (www.shcs.ch) is a prospective cohort study with ongoing enrollment of HIV-infected adults in Switzerland since 1988¹³. It includes 73% of all diagnosed HIV-infections in Switzerland¹⁴. Representativeness has remained stable over the years. Detailed information on demographics, mode of HIV acquisition, risk behavior, clinical events, coinfections, and treatment is collected using a standard protocol at registration and at intervals of 6 months.

Plasma samples are collected every 6-12 months in all study participants. Local ethical committees of all participating study sites have approved the study and written consent has been obtained from all participants.

Inclusion criteria, definitions and outcomes:

All HIV-infected adults with a positive HBsAg test between January 1988 and December 2014 were considered. We performed a screening HDV serology in all patients without information on HDV infection in the database and excluded patients without available stored samples from our analysis. This measurement was performed on the stored serum samples drawn closest to the diagnosis of HBV infection. In all individuals with a positive HDV serology, we quantitatively assessed HDV RNA in the same sample or the first available sample after the positive HDV serology. To rule out false-positive HDV screening results, we performed a second anti-HDV serology in all patients with negative HDV RNA. Patients with false-positive HDV screening results were re-classified as HDV-negative.

Our primary outcome was overall mortality after the initiation of ART. Individual follow-up started at initiation of ART and ended on the date of death, loss to follow-up or database closure (31.12.2014), whichever happened first. The secondary outcomes were: development of i) HCC and ii) liver-related death, which included death from chronic viral hepatitis, cirrhosis, HCC, acute liver failure and variceal bleeding, as coded in ICD-10. In the SHCS, data on causes of death are collected on standardized case-report forms, using reports from medical hospitalizations to inform and validate the diagnosis (<http://shcs.ch/userfiles/file/documents/CODE.pdf>). Patients who did not initiate ART, defined as a combination of at least three antiretroviral drugs, were excluded from the longitudinal analyses.

Laboratory analyses:

The ETI-AB-DELTA-2™ (Diasorin, Saluggia, Italy), a competition ELISA test, was used to screen for anti-HDV antibodies in all HBsAg-positive individuals. All analyses were performed according to the manufacturer's instructions and the results were considered positive when the optical density (OD) was <0.9. For HDV amplification, total nucleic acids were purified from 200µl plasma (Qiagen EZ1 DSP kit) and cDNA (High Capacity cDNA Reverse Transcription Kit, Applied Biosystems™) was subjected to real-time polymerase chain reaction (PCR) according to Ferns et al., with minor modifications¹⁵. The detection limit of HDV real-time PCR was 1000 genome equivalents per ml of plasma. The HDV genotype was assessed by sequencing and phylogenetic analysis of a 260-base-pair cDNA fragment encompassing the 3' end of the hepatitis D antigen coding region. A second serological ELISA test with a sensitivity and specificity >98% (HDV-Ab™kit, Diapro, Milan, Italy,

performed with an ETI-Max [DiaSorin] platform) was used to confirm the anti-HDV screening results from all samples with a positive screening serology but negative HDV RNA. A result was considered negative when the ratio (threshold/OD) was <0.9 . Plasma samples were diluted 1:1 with plasma and HBV was quantified with the COBAS® TaqMan® HBV Test v2.0 on the COBAS AmpliPrep/TaqMan48 system (Roche Diagnostics International AG, Rotkreuz, Switzerland) according to the manufacturer's protocol. HBV Genotyping was performed by PCR Amplification, Sanger sequencing and subsequent in silico analysis by the geno2pheno tool (<http://hbv.geno2pheno.org/index.php>) as described by Hirzel et al¹⁶. The quantitative determination of HBsAg was analyzed with a fully automated chemiluminescent microparticle immunoassay (Architect, Abbott Diagnostics, USA). The Architect HBs Ag Assay has a sensitivity of ≤ 0.05 IU/ml.

Statistical analyses:

The prevalence of HDV infection among all HIV/HBV-coinfected individuals was estimated after the re-classification of false-positive results and given with a 95% confidence interval (CI). Demographic and clinical characteristics at initiation of ART were described using absolute numbers and proportions, or medians and interquartile ranges (IQR), and compared between HIV/HBV- and HIV/HBV/HDV-coinfected patients using Chi-square, Fisher's exact or Mann-Whitney test, where appropriate. Kaplan-Meier analyses and the rank-sum tests were used to compare overall and liver-related mortality as well as HCC-free survival between study groups. The association between HDV infection and the main outcomes was further explored using multivariable Cox regression analyses adjusted for age, sex, CD4 cell count, stage of HIV infection, most likely source of HIV transmission and hepatitis C virus (HCV) coinfection.

We performed several sensitivity analyses. We repeated the main analysis comparing HIV/HBV/HDV-RNA negative and HIV/HBV/HDV-RNA positive patients. We evaluated the association between HDV infection and the main outcomes after the exclusion of persons who inject drugs (PWID) and patients with a positive HCV serology. As these two categories of patients are more likely to have multiple comorbidities and to die during follow-up, their over-representation in the HDV-positive group could have had an important impact on our results. As longitudinal data on HBV replication were lacking, we used TDF treatment as a proxy for HBV suppression, and evaluated the association between HDV infection and the main outcomes after the exclusion of persons who had never been treated with TDF. All statistical analyses were performed using Stata Version 13.1. (StataCorp 2012, Stata Statistical Software, College Station, TX, USA).

Results:

HDV prevalence

Of 818 patients with a positive HBsAg, 771 (94%) had an HDV serology performed, either during routine care (422/771) or from a stored serum sample (349/771) (**Fig. 1**). Overall, 139 patients (18%) had a positive HDV screening serology, of which 122 had a sample available for amplification. Seventy-three patients (59%) examined by PCR had a detectable HDV RNA. After the re-classification of 20 patients with a positive HDV screening test but negative viral load and negative confirmation anti-HDV tests, the prevalence of HDV infection was 15.4% (119/771, 95% CI: 12.9-18.0) and the proportion of HDV-positive patients with HDV replication 62.9% (73/116). Of 70 patients with available results from HDV sequencing, 66 (94.3%) were infected with genotype 1 and four (5.7%) with genotype 5.

Characteristics of HDV infection

Fifteen (12.6%) patients from the HDV-positive group and 29 (4.5%) from the HDV-negative group never started ART during follow-up and were excluded from the longitudinal analyses. **Table 1** summarizes the main characteristics of the study population at ART initiation, by HDV-infection status. Whereas age and sex distribution were similar across both groups, HDV-positive individuals were more likely to be PWID (60.6% vs. 9.1%) and to have a positive HCV serology (73.1% vs. 17.8%) compared to HDV-uninfected ones. Among individuals with a positive HCV serology, only 15/76 (20%) in the HDV-positive group had detectable HCV RNA compared to 67/111 (60%) in the HDV-uninfected group. In HIV/HBV/HDV-coinfected patients with replicating HDV, HCV-RNA was suppressed in 90% of patients. Of the anti-HDV-positive patients, 34/74 (46%) had suppressed HBV DNA at the time of measurement and 75% of them were under an ART with at least one drug active against HBV. Compared to HDV-negative patients, patients coinfecting with HDV were more likely to have genotype D HBV infection. HDV-RNA-positive patients were more likely to have a replicating HBV-DNA and had higher quantitative HBsAg than HDV-RNA-negative patients (**Table S3**). The median CD4 cell count as well as the relative CD4 value were lower in HDV-infected (180 vs. 248 cells/ μ l and 19% vs. 24%) and the median ALT level higher (61 vs. 33 IU/L) than in HDV-negative patients.

Mortality and liver related outcomes

Thirty-six (34.6%) HDV-infected patients and 96 (15.4%) HDV-uninfected individuals died during 6,718 patient-years (py) of follow-up. Median follow-up was 8.71 years (interquartile range [IQR]: 5.01-13.78) in the HDV-positive and 9.75 years (IQR: 4.71-15.27) in the HDV-negative group. Causes of death by HDV status are summarized in **Fig. 2**. One half of the deaths were liver-related (18/36) in HDV-infected patients (8 deaths from HCC and 10 from

decompensated liver disease), compared to 24.0% (23/96) in HDV-uninfected individuals (12 deaths from HCC and 11 from decompensated liver disease). Overall mortality was higher in HDV-infected patients (3.62 deaths per 100 py, 95% CI 2.59-5.07) compared to uninfected ones (1.49 per 100 py, 95% CI 1.20-1.84). Kaplan-Meier cumulative survival probability was significantly higher among HDV-negative patients ($p < 0.001$, **Fig. 3A**). The cumulative probability of liver-related deaths as well as HCC were significantly higher in HDV-positive patients compared to HDV-negative ones (both $p < 0.001$; **Fig. 3B and 3C**).

In multivariable analyses, HDV infection was strongly associated with overall death (adjusted hazard ratio [aHR] 2.33, 95% CI 1.41-3.84), with liver-related mortality (aHR 7.71, 95% CI 3.13-18.97), and with the occurrence of HCC (aHR 9.30, 95% CI 3.03-28.61, **Fig. 4** and **Table S1**). The risk for non-liver related deaths did not differ significantly between HDV-positive and -negative patients (aHR 1.36, 95% CI 0.72-2.57). In analyses comparing HDV-RNA-negative and HDV-RNA-positive patients, we found that the cumulative probability for overall death, liver-related death and HCC were significantly higher in HDV-RNA-positive patients (**Table S2, Fig. 3**, panels D-F). In multivariable analyses, HDV replication was strongly associated with overall death (adjusted hazard ratio [aHR] 7.14, 95% CI 1.60-31.85), liver-related mortality and the occurrence of HCC. There were no liver-related deaths and no HCC in patients that were HDV-RNA-negative. Results were similar when PWID or HCV-infected individuals were excluded from the analyses (**Fig. 4**). In analyses stratified by viral hepatitis coinfection sub-group (HIV/HBV, HIV/HBV/HDV, HIV/HBV/HCV and HIV/HBV/HDV/HCV), overall survival was lowest in the two groups of HDV-infected individuals, independently of anti-HCV antibody status or replicating HCV infection (**Fig. S2 and S3**). In analyses restricted to patients who had received TDF (77%), the point estimate of the association between HDV infection and overall death remained similar (aHR 2.03, 95% CI 0.80-5.15).

Discussion:

The prevalence of HDV infection was high among HIV/HBV-coinfected individuals in Switzerland: 15% were antibody-positive and 63% of them had detectable viral replication. Over nine years of individual follow-up on ART, HDV-infected patients were twice as likely to die as HDV-uninfected ones. The excess mortality in HDV-positive patients was driven by liver related deaths: HDV-positive individuals were eight times more likely to die from liver disease and nine times more likely to develop an HCC compared to HDV-negative patients. These associations were robust, remaining similar in all sensitivity analyses.

The prevalence of HDV infection in our cohort was similar to the findings from the EuroSIDA collaboration but slightly higher than the estimate reported in cohort studies from France and

North America^{17,18}. As false-positive HDV screening results seem to be frequent in the setting of HIV-infection¹⁹, we performed a confirmatory serology in all patients with a negative HDV RNA in order to strengthen our HDV prevalence estimate. In our study, 14% of positive HDV screening tests were false-positive. After the exclusion of these patients, 63% of HDV-positive patients had a detectable HDV viral load, which was lower than the proportion found in the EuroSIDA study⁴. Although the main reason for this difference remains unclear, the unselected nature of our study population, in which all patients were screened for HBV and HDV infection independently of clinical manifestations or laboratory abnormalities, might have played an important role. HDV-infected individuals were predominantly PWID of Western European origin, which explains the high proportion of HDV genotype 1 found in our cohort. As all remaining patients (6%) were HDV genotype 5-infected migrants from sub-Saharan Africa diagnosed during the last five years, our results suggest a changing pattern in HDV epidemiology in Switzerland^{2,20,21}. Injection drug use being the most probable route of HDV transmission for the majority of patients included in our study, HCV infection was also present in many of them. The complex interplay between HBV, HCV and HDV has been described in several previous reports which generally showed HDV to be the dominant virus, often leading to the suppression of HBV as well as HCV^{17,22,23}. In line with these findings, only 20% of HIV/HBV/HDV-coinfected patients and 10% of HIV/HBV/HDV-coinfected patients with replicating HDV in our cohort had a replicating HCV infection compared to 60% of HDV-negative individuals. HCV-coinfection had no impact on overall survival in our study. Almost one half of HDV-positive patients had a suppressed HBV-viremia. Those with a replicating HDV were more likely to also have a replicating HBV as well as higher HBsAg titers.

HDV-positive individuals were 2.3 times more likely to die during follow-up on ART than uninfected ones. A detailed analysis of causes of death showed that this finding was driven by the excess liver-related mortality seen in HDV-positive individuals: 50% of HDV-positive patients died of liver disease, resulting in an 8-fold risk increase compared to HDV-negative patients. The impact of HDV on overall and liver-related mortality was further demonstrated by the similar estimates obtained after the exclusion of HCV-infected patients and in analyses limited to patients with optimal HBV-therapy. In a case-control study among HIV-infected patients in Taiwan, Sheng et al. found that HBV/HDV-coinfected individuals were five times more likely to die than HBV-infected ones¹⁰. More recently, Fernandez-Montero et al. confirmed this finding and described an increase in liver-related mortality in HDV-positive individuals comparable to the estimate of the present study⁹. In our study the hazard of death, liver-related death and HCC was mainly driven by HDV-RNA-positive patients. However, due to the low number of HDV-infected patients included in previous studies of

HIV-positive individuals, data on the impact of HDV replication on liver-related outcomes and mortality are scarce.

HCC was diagnosed in 23 patients of which 20 died during the study period. HDV-positive individuals were 9 times more likely to develop HCC as compared to HDV-uninfected HBV positive persons. Chronic HBV infection is the single most important risk factor of HCC worldwide²⁴⁻²⁶, and this risk has been shown to correlate with HBV replication²⁷. However, the impact of HDV on liver carcinogenesis and its contribution to global HCC epidemiology remains unclear. In cirrhotic HBV-infected patients, Fattovich et al. found a 3-fold increase in HCC incidence among HDV-infected patients, whereas similar rates of HCC were found in HBV- and HBV/HDV-infected patients in a retrospective study from London.^{20,28} In Italy, Romeo et al. showed that persistent HDV replication was strongly associated with the development of HCC but no HIV-infected individuals were included⁸. To our knowledge, our study is the first to be powered to detect a difference in HCC incidence between HDV-infected and -uninfected HIV-positive individuals and calls for other cohorts to confirm these results.

We studied the impact of HDV infection on clinical outcomes in one of the largest studies of HIV/HBV-coinfected patients to date. Long individual follow-up time as well as the systematic ascertainment of death and liver-related complications allowed us to provide robust estimates of liver-related mortality and HCC. Only very few patients were excluded from our analyses: HDV serology results were available for 94% of HBsAg-positive patients and 88% of HDV-positive individuals had an assessment of viral replication. Furthermore, the probability of false-positive results was substantially reduced by using confirmatory serologic tests. Unfortunately, information on HBV viral load and genotypes was only available for a limited number of patients, precluding its use in the main analyses. As there is a clear link between HBV viral load and liver-related outcomes such as HCC, our analyses do not provide the full picture of risk factors for liver disease. However, analyses which were restricted to patients on TDF, a good proxy for HBV suppression, showed results which were comparable to the main analyses. Although Su et al. showed worse outcomes in HBV genotype C than B in Taiwan, our results are unlikely to be affected by the HBV genotype distribution, as only 5% of our patients were of Asian origin²⁹. The large difference in the proportion of PWID and HCV infections across both study groups was another limitation of our study, as these characteristics are known risk factors for mortality and liver-related morbidity. Again, sensitivity analyses showed that the association between HDV infection and the main outcomes were independent of these two cofactors. Finally, we only had information on HDV viral replication at one time-point for each patient and, as a

consequence, might have misclassified patients as HDV RNA-negative despite potential subsequently detectable replication.

In conclusion, we found a high prevalence of hepatitis delta in HIV/HBV-coinfected individuals in Switzerland. The strong association between HDV infection and mortality as well as liver-related events, including HCC, highlights the importance of HDV screening of all HIV/HBV-coinfected patients. Our results emphasize the need for systematic screening for HDV infection, the close monitoring of HDV-infected patients and underline the importance of developing and evaluating new treatments for chronic hepatitis D. As potent treatment options for HDV are still lacking, preventing infections through the implementation of efficient HBV vaccination programs remains a cornerstone of the prevention of HDV infection. In persons with HDV-coinfection and ESLD or HCC, liver transplantation from an HBsAg-negative donor should be considered.

Fig. 1: Patient flowchart

Abbreviations: HDV, hepatitis D virus; PCR, polymerase chain reaction; ART, antiretroviral therapy

Table 1: Demographic and clinical characteristics of patients at ART initiation, by HDV status

Abbreviations: ART, antiretroviral therapy; HDV, hepatitis D virus; HBV, hepatitis B virus; IQR, interquartile range; HIV, human immunodeficiency virus; MSM, men who have sex with men; PWID, people who inject drugs; HET, heterosexual; CDC: Centers for Diseases Control; eGFR, estimated glomerular filtration rate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; Ab: antibody; APRI: AST-to-platelet ratio index

*eGFR: calculated with the CKD-EPI formula

**Anemia defined as follows: Mild: hemoglobin <13 G/L in males and <12 G/L in females. Moderate-severe: hemoglobin <11 G/L in males and <10 G/L in females

***ACTG (AIDS Clinical Trials Group) Grade 1-4

Fig. 2: Main cause of death, by hepatitis D virus (HDV) status

Absolute numbers of death are shown at the bottom of the bars

Abbreviations: HIV, human immunodeficiency virus; CVD cardiovascular disease; HDV, hepatitis delta virus

Fig. 3: Kaplan–Meier survival analyses by hepatitis D virus (HDV) serological and replication status for overall survival (A, D), liver-related mortality (B, E) and hepatocellular carcinoma (HCC) free survival (C, F)

Abbreviations: ART, antiretroviral therapy; HDV, hepatitis D virus; CI, confidence interval; HCC, hepatocellular carcinoma

Fig. 4: Forest plot of hazard ratios for the main outcomes (adjusted for age, sex, CD4 cell count, stage of HIV infection, most likely source of HIV transmission and hepatitis C virus (HCV) coinfection)

Abbreviations: aHR, adjusted hazard ratio; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; PWID, people who inject drugs

Acknowledgments

This study has been financed within the framework of the Swiss HIV Cohort Study, supported by the Swiss National Science Foundation (grant #148522), by SHCS project #769 and by the SHCS research foundation. The data are gathered by the Five Swiss University Hospitals, two Cantonal Hospitals, 15 affiliated hospitals and 36 private physicians (listed in <http://www.shcs.ch/180-health-care-providers>).

Members of the Swiss HIV Cohort Study

Aubert V, Battegay M, Bernasconi E, Böni J, Braun DL, Bucher HC, Burton-Jeangros C, Calmy A, Cavassini M, Dollenmaier G, Egger M, Elzi L, Fehr J, Fellay J, Furrer H (Chairman of the Clinical and Laboratory Committee), Fux CA, Gorgievski M, Günthard H (President of the SHCS), Haerry D (deputy of "Positive Council"), Hasse B, Hirsch HH, Hoffmann M, Hösli I, Kahlert C, Kaiser L, Keiser O, Klimkait T, Kouyos R, Kovari H, Ledergerber B, Martinetti G, Martinez de Tejada B, Marzolini C, Metzner K, Müller N, Nadal D, Nicca D, Pantaleo G, Rauch A (Chairman of the Scientific Board), Regenass S, Rudin C (Chairman of the Mother & Child Substudy), Schöni-Affolter F (Head of Data Centre), Schmid P, Speck R, Stöckle M, Tarr P, Trkola A, Vernazza P, Weber R, Yerly S.

References:

1. Rizzetto M, Ponzetto A, Forzani I. Hepatitis delta virus as a global health problem. *Vaccine*. 1990;8 Suppl:S10-14; discussion S21-13.
2. Wedemeyer H, Heidrich B, Manns MP. Hepatitis D virus infection--not a vanishing disease in Europe! *Hepatology*. 2007;45(5):1331-1332; author reply 1332-1333.
3. Hughes SA, Wedemeyer H, Harrison PM. Hepatitis delta virus. *Lancet*. 2011;378(9785):73-85.
4. Soriano V, Grint D, d'Arminio Monforte A, et al. Hepatitis delta in HIV-infected individuals in Europe. *Aids*. 2011;25(16):1987-1992.
5. Hønge BL, Jespersen S, Medina C, et al. Hepatitis B and Delta virus are prevalent but often subclinical co-infections among HIV infected patients in Guinea-Bissau, West Africa: a cross-sectional study. *PLoS One*. 2014;9(6):e99971.
6. Gaeta GB, Stroffolini T, Chiaramonte M, et al. Chronic hepatitis D: a vanishing Disease? An Italian multicenter study. *Hepatology*. 2000;32(4 Pt 1):824-827.
7. Saracco G, Rosina F, Brunetto MR, et al. Rapidly progressive HBsAg-positive hepatitis in Italy. The role of hepatitis delta virus infection. *J Hepatol*. 1987;5(3):274-281.
8. Romeo R, Del Ninno E, Rumi M, et al. A 28-year study of the course of hepatitis Delta infection: a risk factor for cirrhosis and hepatocellular carcinoma. *Gastroenterology*. 2009;136(5):1629-1638.
9. Fernandez-Montero JV, Vispo E, Barreiro P, et al. Hepatitis delta is a major determinant of liver decompensation events and death in HIV-infected patients. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2014;58(11):1549-1553.
10. Sheng WH, Hung CC, Kao JH, et al. Impact of hepatitis D virus infection on the long-term outcomes of patients with hepatitis B virus and HIV coinfection in the era of highly active antiretroviral therapy: a matched cohort study. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2007;44(7):988-995.
11. Soriano V, Vispo E, Sierra-Enguita R, et al. Efficacy of prolonged tenofovir therapy on hepatitis delta in HIV-infected patients. *Aids*. 2014;28(16):2389-2394.
12. Boyd A, Miallhes P, Brichler S, et al. Effect of tenofovir with and without interferon on hepatitis D virus replication in HIV-hepatitis B virus-hepatitis D virus-infected patients. *AIDS Res Hum Retroviruses*. 2013;29(12):1535-1540.

13. Swiss HIVCS, Schoeni-Affolter F, Ledergerber B, et al. Cohort profile: the Swiss HIV Cohort study. *Int J Epidemiol*. 2010;39(5):1179-1189.
14. Kohler P, Schmidt AJ, Cavassini M, et al. The HIV care cascade in Switzerland: reaching the UNAIDS/WHO targets for patients diagnosed with HIV. *Aids*. 2015;29(18):2509-2515.
15. Ferns RB, Nastouli E, Garson JA. Quantitation of hepatitis delta virus using a single-step internally controlled real-time RT-qPCR and a full-length genomic RNA calibration standard. *J Virol Methods*. 2012;179(1):189-194.
16. Hirzel C, Wandeler G, Owczarek M, et al. Molecular epidemiology of hepatitis B virus infection in Switzerland: a retrospective cohort study. *BMC Infect Dis*. 2015;15:483.
17. Boyd A, Lacombe K, Miallhes P, et al. Longitudinal evaluation of viral interactions in treated HIV-hepatitis B co-infected patients with additional hepatitis C and D virus. *J Viral Hepat*. 2010;17(1):65-76.
18. Kushner T, Serper M, Kaplan DE. Delta hepatitis within the Veterans Affairs medical system in the United States: Prevalence, risk factors, and outcomes. *J Hepatol*. 2015;63(3):586-592.
19. Winter A, Letang E, Kalinjuma AV, et al. Absence of Hepatitis Delta Infection in a Large Rural HIV Cohort in Tanzania. *Int J Infect Dis*. 2016.
20. Cross TJ, Rizzi P, Horner M, et al. The increasing prevalence of hepatitis delta virus (HDV) infection in South London. *J Med Virol*. 2008;80(2):277-282.
21. Kucirka LM, Farzadegan H, Feld JJ, et al. Prevalence, correlates, and viral dynamics of hepatitis delta among injection drug users. *The Journal of infectious diseases*. 2010;202(6):845-852.
22. Mathurin P, Thibault V, Kadidja K, et al. Replication status and histological features of patients with triple (B, C, D) and dual (B, C) hepatic infections. *J Viral Hepat*. 2000;7(1):15-22.
23. Arribas JR, Gonzalez-Garcia JJ, Lorenzo A, et al. Single (B or C), dual (BC or BD) and triple (BCD) viral hepatitis in HIV-infected patients in Madrid, Spain. *Aids*. 2005;19(13):1361-1365.
24. Beasley RP. Hepatitis B virus. The major etiology of hepatocellular carcinoma. *Cancer*. 1988;61(10):1942-1956.
25. Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J Hepatol*. 2006;45(4):529-538.
26. Moradpour D, Blum HE. Pathogenesis of hepatocellular carcinoma. *Eur J Gastroenterol Hepatol*. 2005;17(5):477-483.
27. Chen CJ, Yang HI, Su J, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA*. 2006;295(1):65-73.
28. Fattovich G, Giustina G, Christensen E, et al. Influence of hepatitis delta virus infection on morbidity and mortality in compensated cirrhosis type B. The European Concerted Action on Viral Hepatitis (Eurohep). *Gut*. 2000;46(3):420-426.
29. Su CW, Huang YH, Huo TI, et al. Genotypes and viremia of hepatitis B and D viruses are associated with outcomes of chronic hepatitis D patients. *Gastroenterology*. 2006;130(6):1625-1635.

Hepatitis delta virus (HDV) infection accelerates the progression of hepatitis B virus (HBV)-related liver disease. In a nationwide cohort of HIV-infected individuals in Switzerland, 15% of HBV-coinfected patients had antibodies to HDV infection, of which a majority had active HDV replication. HDV-infected individuals were 2.5 times more likely to die, 8 times more likely to die from a liver-related cause and 9 times more likely to develop liver cancer compared to HDV-uninfected ones. Our results emphasize the need for prevention programs (including HBV vaccination), the systematic screening of at risk populations as well as close monitoring, and underline the importance of developing new treatments for chronic HDV infection.

Table 1: Demographic and clinical characteristics of patients at ART initiation, by HDV status

Characteristics	HDV-positive (N=104)	HDV-negative (N=623)	P
Age, median (IQR)	34 (29-37)	36 (30-43)	<0.001
Male sex (%)	79 (76.0)	487 (78.2)	0.25
Region of origin (%)			0.01
Northwestern Europe	74 (71.1)	344 (55.2)	
Central and Eastern Europe	2 (1.9)	18 (2.9)	
Southern Europe (Mediterranean)	14 (13.5)	64 (10.3)	
Sub-Saharan Africa	13 (12.5)	131 (21)	
Southeast Asia	1 (1)	38 (6.1)	
Other	0 (0)	28 (4.5)	
Education level (%)			<0.001
No or low	49 (47.1)	187 (30)	
Medium	35 (33.6)	255 (40.9)	
High	7 (6.7)	150 (24.1)	
No information	13 (12.5)	31 (5)	
HIV transmission group (%)			<0.001
MSM	10 (9.6)	300 (48.1)	
PWID	63 (60.6)	57 (9.1)	
HET	17 (16.3)	217 (34.8)	
Other	14 (13.5)	49 (7.9)	
Median HIV RNA in log cp/ml (IQR)	4.6 (3.2-5.1)	4.7 (3.3-5.2)	0.70
CDC stage C (%)	34 (32.7)	191 (30.7)	0.17
Median CD4 count in cells/μl (IQR)	180 (96-314)	248 (120-358)	0.03
CD4 cell count category in cells/μl (%)			0.07
1-200	52 (50.5)	242 (40)	
201-350	33 (32)	201 (33.2)	
>350	18 (17.5)	162 (26.8)	
Median creatinine in μmol/L (IQR)	71.5 (67-84)	75 (65-90)	0.68
eGFR in ml/min (IQR)*	103 (94-123)	109 (93-126)	0.80
Median hemoglobin in g/L (IQR)	13.8 (12.4-14.2)	14.1 (12.7-15.2)	0.11
Anemia (%)**			0.66
No	72 (75.8)	415 (76.4)	
Mild	16 (16.8)	100 (18.4)	
Moderate-Severe	7 (7.4)	28 (5.2)	
Median thrombocytes in G/L (IQR)	158 (113-212)	206 (161-246)	<0.001
Median ALT in IU/L (IQR)	61 (36-90)	33 (23-59)	0.04
Anti-HCV positive (%)	76 (73.1)	111 (17.8)	<0.001

Abbreviations: ART, antiretroviral therapy; HDV, hepatitis D virus; HBV, hepatitis B virus; IQR, interquartile range; HIV, human immunodeficiency virus; MSM, men who have sex with men; PWID, people who inject drugs; HET, heterosexual; CDC: Centers for Diseases Control ; eGFR, estimated glomerular filtration rate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; Ab: antibody; APRI: AST-to-platelet ratio index

*eGFR: calculated with the CKD-EPI formula

**Anemia defined as follows: Mild: hemoglobin <13 G/L in males and <12G/L in females. Moderate-severe: hemoglobin <11 G/L in males and <10G/L in females

***ACTG (AIDS Clinical Trials Group) Grade 1-4

Figure 1

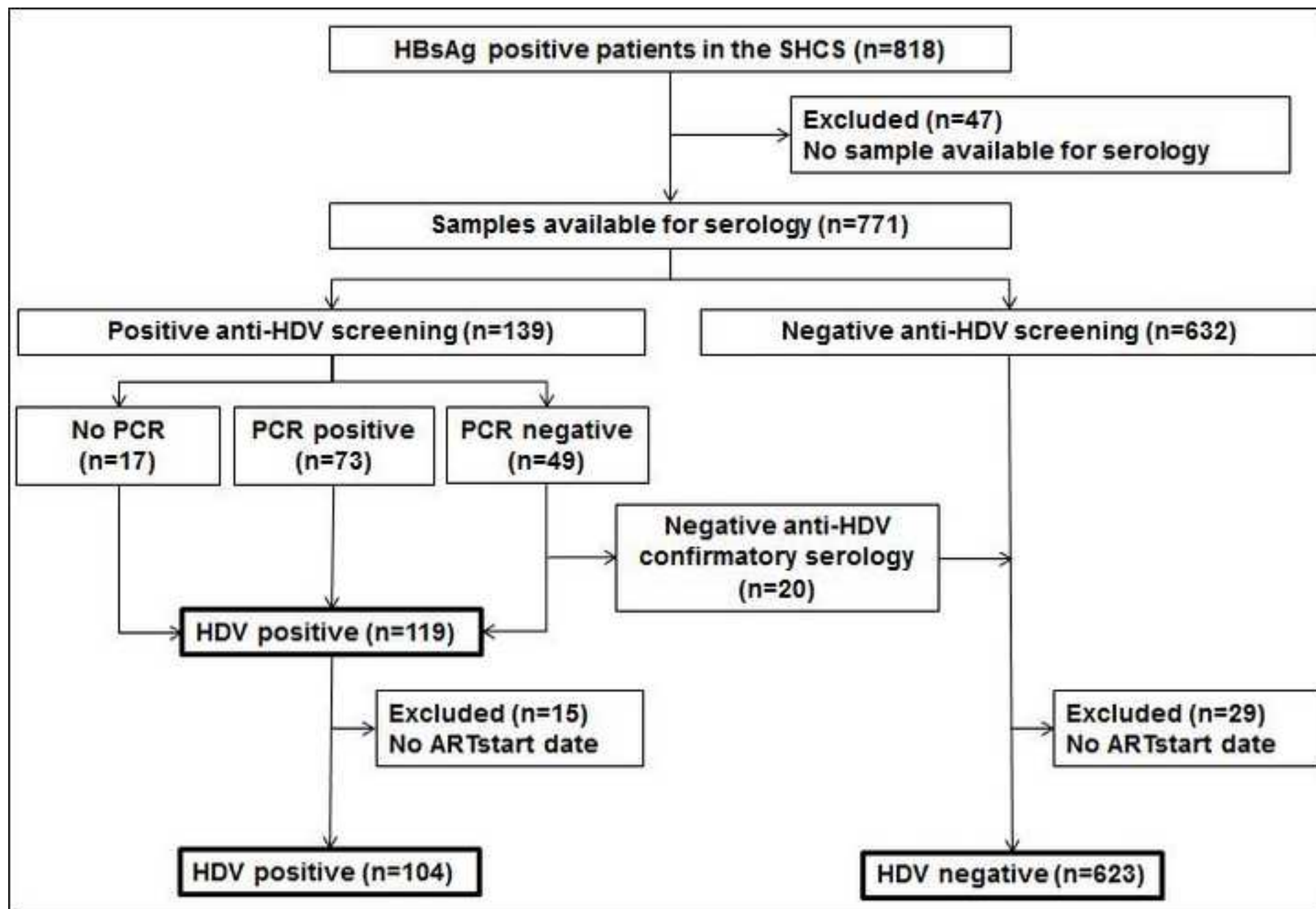


Figure 2

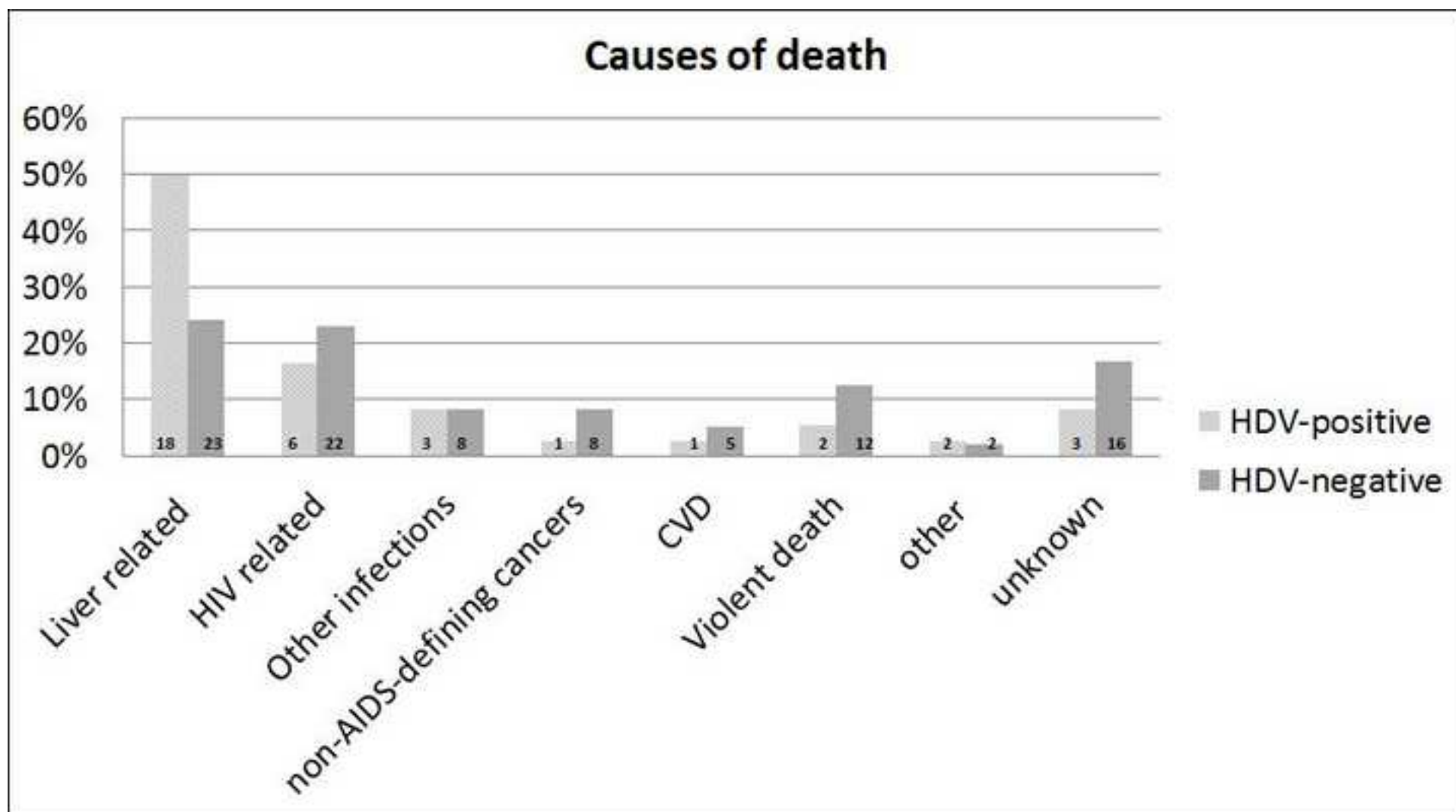
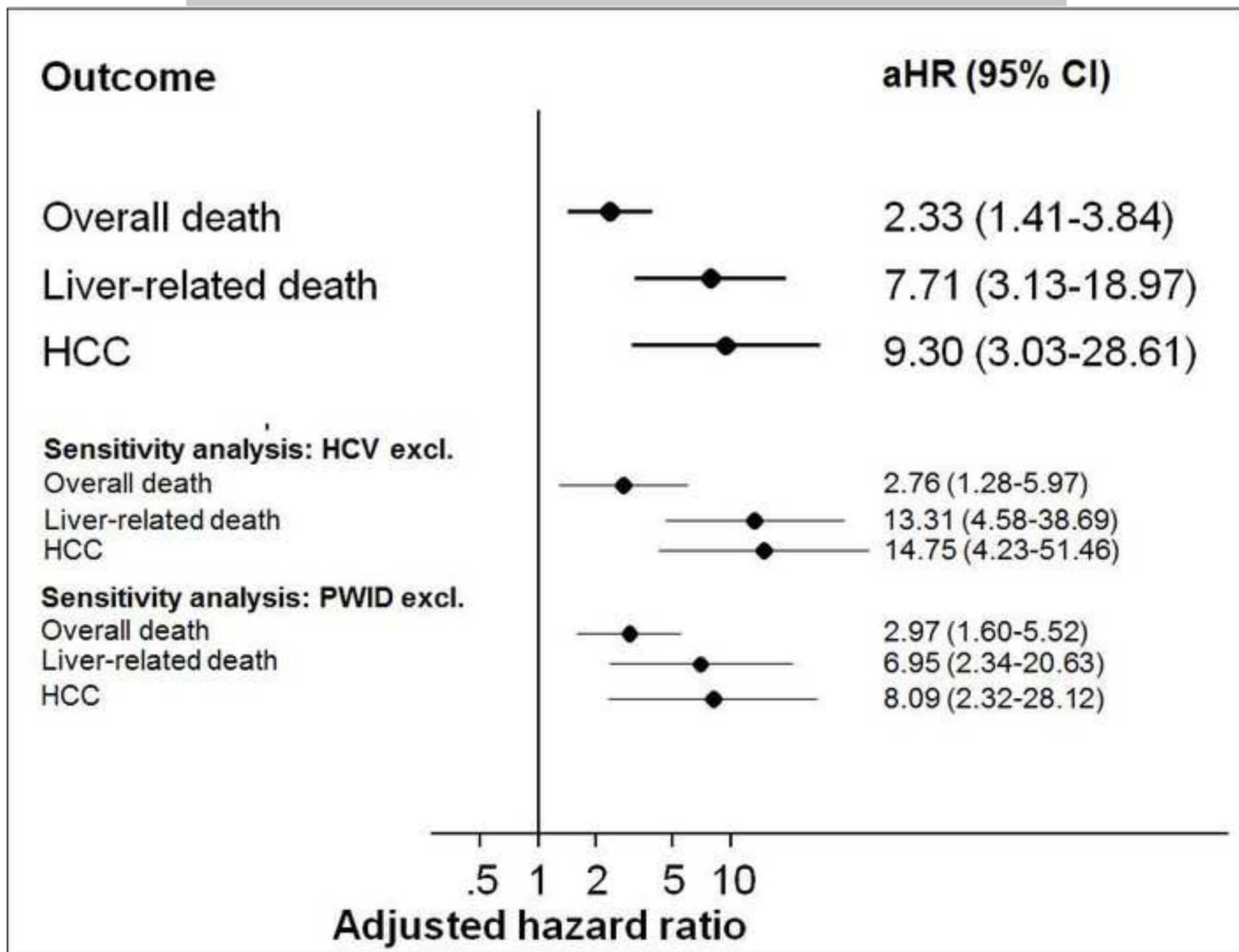
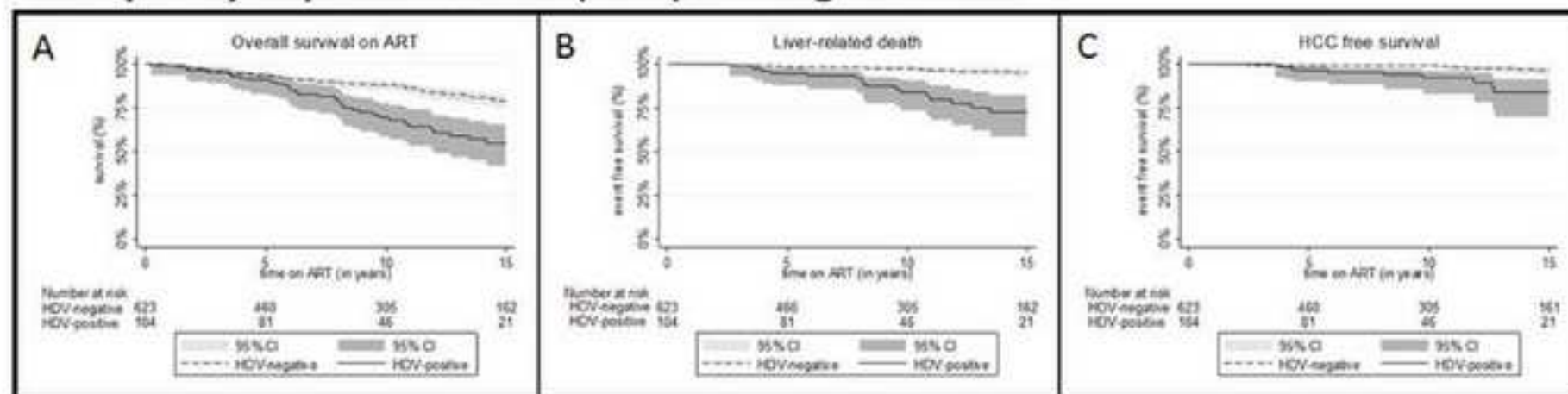


Figure 4



Analysis by hepatitis D virus (HDV) serological status



Analysis by HDV-RNA status in anti-HDV seropositive patients

